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"PAPOID," a therapeutic agent, noted especially for its peculiar proteolytic power, is prepared from various parts of the papaw plant, *Carica Papaya*. As pointed out by Martin,* the occurrence of such a proteolytic ferment in the vegetable kingdom is in itself remarkable, and its discovery, together with that of other vegetable ferments, may eventually throw important light on the assimilation of animal food by carnivorous plants, as well as on the character of the proteolytic changes in the reserve proteids of plants in general.

Furthermore, such vegetable ferments when properly isolated may prove of great value in medicine as therapeutic agents, and it is with this point in view that the following study of papoid has been undertaken.

The researches of Martin† have shown that papaw juice is peculiarly rich in a variety of proteids, with one or more of which the proteolytic ferment is associated. Hence, it would naturally be expected that any active preparation of this ferment would contain a large proportion of proteid or albuminous material. This is the case with papoid, and the proteids present, as seen from the following reactions, are of several kinds.

1. *General Reactions of Papoid.*

Papoid, treated with distilled water, yields on filtration, a yellowish colored solution, leaving a small, flocky, insoluble residue. The solution is almost neutral, showing, however, a faint alkaline reaction when tested carefully. A drop or two of dilute acid does not, however, give any neutralization precipitate. The matter insoluble in water is partially dissolved by a 5 per cent. solution of sodium chloride, the fluid giving a fairly heavy precipitate with concentrated nitric acid, which on heating turns yellow, but does not dissolve, thus indicating the presence of a *globulin*. This residue of globulin is also soluble in 0·5 per cent. sodium carbonate and in 0·2 per cent. hydrochloric acid, from both of which solutions it is reprecipitated by neutralization, again dissolving in a slight excess of either dilute acid or alkali. That portion of the residue not dis-

* Journal of Physiology, vol. 5, p. 213. † Ibid., vol. 6, p. 341.

solved by salt solution, dilute acid or alkali, is composed mainly of insoluble or *coagulated* proteid.

The addition of distilled water to a clear, aqueous solution of papoid produces a pronounced turbidity, which disappears at once on the addition of a little salt solution, thus showing the presence of a globulin which is obviously held in solution by virtue of the salts contained in the preparation.

Concentrated hydrochloric acid added to a clear, aqueous solution of papoid gives a heavy white precipitate of proteid matter, readily soluble in excess of the strong acid.

Concentrated nitric acid, under similar circumstances, produces a heavy white precipitate, which on heating or boiling dissolves in great part, yielding a reddish-yellow solution. On cooling this solution, the precipitate reappears, dissolving again as the mixture is heated, and once more reappearing as the fluid cools. This reaction is due to the presence of an *albumose*, while the accompanying body precipitated by nitric acid, but insoluble on heating, is a *globulin*, presumably the same as that previously noted in the insoluble matter.

Acetic acid and potassium ferrocyanide produce a heavy white precipitate only in part dissolved by warming.

Boiling a clear, aqueous solution of papoid gives rise to a turbidity, which on prolonged boiling passes into a flocculent precipitate. This precipitate is insoluble in 0·2 per cent. hydrochloric acid, and is therefore presumably composed of *coagulated globulin.*

Addition of magnesium sulphate in substance to an aqueous solution of papoid precipitates the globulin present, with perhaps some albumose. At first sight, from the milky appearance of the fluid, the precipitate appears quite voluminous, but the amount is in reality not large. On adding crystals of sodium sulphate to the filtrate from the above precipitate, in such quantity as to insure complete saturation of the fluid and with formation of sodio-magnesium sulphate, a second heavier precipitate is produced, composed mainly of albumoses (one or more) which agglutinates into a somewhat gummy mass, especially on the addition of a drop or two of acetic acid. With this precipitate of albumose, the proteolytic ferment appears to be mainly associated.

In the filtrate from this second or sodio-magnesium sulphate precipitate, the presence of peptone, in considerable quantity, can be shown by the biuret test. Or, by directly saturating an aqueous solution of papoid with ammonium sulphate while warm, the albu-

moses and globulin can be precipitated together, while the peptone can be detected in the filtrate by the biuret test, viz: by potassium hydroxide* and dilute cupric sulphate solution.

From the foregoing simple reactions, it is evident that papoid is composed essentially of a mixture of globulin, albumoses and peptone, with which is associated the ferments characteristic of the preparation. This is essentially in accord with what is known regarding the vegetable ferments in general, and indeed, the animal ferments as well. Thus even pepsin, in its general chemical reactions, behaves like an albumose, and the best known methods of isolating vegetable ferments result simply in the separation of one or more albumoses, or a glodulin-like albumose, with which the ferment appears to be inseparably connected. In addition to the above proteid constituents, papoid appears to contain a small amount of indifferent material, probably added to counteract any tendency which the peptones or other like bodies have towards the accumulation of hygroscopic moisture.

2. *The Proteolytic Action of Papoid.*

As early as 1874, Roy† had called attention to the fact that papaw juice had the power of dissolving both animal and vegetable albumin, although he apparently did not clearly recognize the process as one of digestion. Later, Albrecht‡ experimented in the same direction, and since then many experimenters have added their testimony to the power of papaw preparations as solvents for proteid matter.

Papoid, so far as my observations extend, has the power of digesting to a greater or less extent all forms of proteid or albuminous matter, both coagulated and uncoagulated. Furthermore, papoid is peculiar in that its digestive power is exercised in a neutral, acid and alkaline medium. These statements are amply illustrated by the following experiments :

a. Action on Coagulated Egg-albumin.

The albumin was prepared for this experiment by taking the whites of several eggs, cutting the transparent membranes with scissors, adding an equal volume of distilled water and straining the mixture through fine muslin to remove the meshes enclosing the

* The potassium hydroxide solution must be added in large excess, sufficient to decompose all of the ammonium salt present.

† Glasgow Med. Journal, 1874. ‡ Schmidt's Jahrbuch, vol. 190.

albumin. The so-prepared solution was then poured, with constant stirring, into a comparatively large volume of boiling water acidified with acetic acid. By this treatment the albumin was coagulated in fairly fine flocks, after which it was collected on a cloth filter, washed thoroughly with boiling water and pressed as dry as possible.

The digestions were carried out as follows: each digestive mixture contained 0·5 gram papoid, 10 grams of the moist coagulated egg-albumin, and 25 c. c. of water in which were dissolved the necessary amounts of alkali or acid to give the indicated percentages. All the mixtures of the series were placed in a water-bath kept at a temperature of 40–45° C., where they were allowed to stand for 12 hours with frequent stirring. At the end of this time, the undigested residues were filtered off on weighed filters of pure washed Swedish filter paper, which had been previously dried at 110° C. in suitable weighing bottles. The undigested residues were then washed with hot water until all soluble products were removed, after which they were dried at 110° C. until of constant weight.

To each digestive mixture was added, likewise, eight drops of an alcoholic solution of thymol, in order to prevent possible putrefaction.

The 10 grams of moist coagulated egg-albumin used in each digestion contained 1·5143 grams of dry proteid, as was ascertained by drying a portion at 110° C. until of constant weight.

The following figures show the results obtained:

Reaction.	Weight of undigested residue.	Coagulated albumin digested.
Neutral	0·6503 gram.	57·0 per cent.*
0·05 per cent. Hydrochloric acid	0·6297 "	58·4 "
0·10 " " "	0·9060 "	40·1 "
2·00 " Bicarb. soda	0·4144 "	72·6 "
4·00 " " "	0·3896 "	74·2 "

Without papoid, the above percentages of acid and alkali have little action on coagulated egg-albumin, as is seen from the two following experiments which were carried out exactly like the foregoing, omitting only the papoid.

Reaction.	Weight of undissolved residue.	Coagulated albumin dissolved.
0·1 per cent. Hydrochloric acid	1·4990 grams.	1·0 per cent.
4·0 " Bicarb. soda	1·4684 "	3·0 "

* Calculated on the amount of dry albumin contained in the 10 grams of moist coagulum.

From these results, it is plain that papoid will digest coagulated egg-albumin in neutral, acid and alkaline solutions; its solvent power being most marked, in this case, in the presence of 2 to 4 per cent. sodium bicarbonate.

In considering these quantitative results, and those which follow, it must be noted that the figures given cannot be taken as an *exact* measure of the extent of digestion, but simply as a measure of the conversion of the insoluble proteid into soluble products. Thus, it may be that the so-called undigested residue consists in some cases, wholly or in part, of alteration products, which though insoluble are still products of the activity of the ferment, while in other cases the residue may consist simply of the unaltered proteid. Discussion of this point, which has a bearing on the *completeness* of papoid digestion, must be reserved until we come to consider the products of digestion.

b. Action on Cooked Beef Proteids.

The beef proteids used in this series of experiments were prepared by taking lean, round steak, passing it through a hashing machine, then washing it repeatedly with water until all blood was removed and it had become nearly white in color. It was then placed in fresh water and heated until the water boiled, after which it was strained off through a cloth filter and pressed as dry as possible.

Each digestive mixture contained 0·5 gram papoid, 10 grams of the cooked beef, and 25 c. c. of water in which were dissolved the indicated percentages of acid and alkali.

The 10 grams of cooked beef contained 3·7438 grams of dry proteid (dried at 110° C.)

The digestions were all warmed at 45° C. for 5½ hours, with frequent stirring, after which the undigested residues were filtered off, washed, dried and weighed as described in the preceding experiment.

Reaction.	Weight of undigested residue.	Cooked beef proteids digested.
Neutral	1·7782 grams.	52·5 per cent.
0·05 per cent. Hydrochloric acid	1·7221 "	54·0 "
0·1 " " "	1·9179 "	48·7 "
0·2 " " "	3·0679 "	18·0 "
2·0 " Bicarb. soda	1·4338 "	61·7 "
4·0 " " "	1·2651 "	66·2 "
Without papoid.		
0·2 " Hydrochloric acid	3·5738 "	4·5 "
4·0 " Bicarb. soda	3·5205 "	5·9 "

From these results, it is manifest that papoid will digest and dissolve cooked beef proteids more readily even than it dissolves coagulated egg-albumin, since the above results were obtained in 5½ hours digestion at 45° C., while the slightly higher results given for the coagulated albumin were obtained after 12 hours digestion at the same temperature. It is further noticeable that the ferment acts most energetically, as in the preceding series of experiments, in the presence of 2–4 per cent. sodium bicarbonate, while a slight addition of acid increases the solvent action a trifle over that of the neutral solution.

c. Action on Raw Beef Proteids.

The beef used in this series of experiments was simply hashed, lean beef, washed with water until it was completely free from all soluble matters and nearly or quite white in color.

Each digestive mixture contained 0·5 gram papoid, 10 grams of the prepared beef, and 25 c. c. of water containing the percentages of acid and alkali indicated.

The 10 grams of beef contained 2·8508 grams of dry proteid (dried at 110° C.)

The digestions were kept at 45° C. for 7 hours.

Reaction.	Weight of undigested residue.	Raw beef proteids digested.
Neutral	0·8988 gram.	68·4 per cent.
0·05 per cent. Hydrochloric acid	0·8910 "	68·7 "
0·10 " " "	1·0901 "	61·7 "
0·20 " " "	2·0145 "	29·3 "
2·00 " Bicarb. soda	good digestion, but the residue was so slimy it could not be filtered.	
4·00 " " "	" "	" "
Without papoid.		
0·2 per cent. Hydrochloric acid	2·5576 grams	10·3 per cent.
2·0 " Bicarb. soda	2·7596 "	3·2 "

Here, as in the preceding experiments, there is evidence of vigorous digestive action especially pronounced in the neutral solution, although still marked in the presence of both dilute acid and alkali. In fact, it is to be presumed, from analogy, that the action of the ferment was even greater in the alkaline solution than in the neutral fluid, although no numerical data were obtained bearing on this point.

d. Action on Raw Blood-fibrin.

Fresh blood-fibrin washed for 18 or 24 hours with cold water until perfectly white, was pressed as dry as possible and cut into moderately small fragments.

Each digestive mixture contained 0·5 gram papoid, 15 grams of moist fibrin, and 50 c. c. of water together with the indicated percentage of acid or alkali.

The 15 grams of fibrin contained 3·4161 grams of dry proteid (110° C.).

The digestions were warmed at 40–45° C. for 8 hours.

Reaction.	Weight of undigested residue.	Raw fibrin digested.
Neutral	1·9077 grams	44·1 per cent.
0·04 per cent. Hydrochloric acid	1·4452 "	57·6 "
1·00 " Bicarb. soda	1·9469 "	43·0 "

A second series of experiments with raw blood-fibrin was tried, especially to ascertain more fully the influence of an increase of acid on the action of the ferment. The results, however, are not only interesting as showing the effect of dilute acid, but are likewise instructive as showing the influence of dilution on the action of the ferment.

Each digestive mixture contained 10 grams of raw fibrin, 0·5 gram papoid, and 25 c. c. of water together with the indicated percentages of acid.

The digestions were continued for 3½ hours at 40–45° C.

The 10 grams of moist fibrin contained 2·277 grams of dry proteid (110° C.).

Reaction.	Weight of undigested residue.	Raw fibrin digested.
Neutral	0·8982 gram	60·5 per cent.
0·04 per cent. Hydrochloric acid	0·2452 "	89·2 "
0·10 " " "	0·5270 "	76·8 "
0·20 " " "	1·6746 "	26·4 "

These two series of experiments agree in showing a very marked increase of digestive action in the presence of small amounts of hydrochloric acid. Furthermore, the last series, especially, shows that raw fibrin is particularly susceptible to the action of papoid, both in a neutral and acid solution. In the last experiment, it is to be noted that the digestions were continued for only 3½ hours, and yet nearly 90 per cent. of the fibrin was dissolved in one case. This is suggestive in view of the fact that raw blood-fibrin probably comes nearest chemically to the so-called pseudo-membranes, such

as are formed in diphtheria, etc., and hence we might fairly draw the deduction that the pseudo-membranes would be attacked by papoid with equal facility.

As already stated, these two series of experiments show well the effect of dilution on the action of papoid as a proteolytic agent. In the first series of experiments, it is to be noted that the total volume of fluid in each digestive mixture was 50 c. c., while in the second series of experiments, only half that volume of fluid was used, the amount of papoid being the same in both cases. Furthermore, in the first series of experiments, the mixtures were warmed at 40–45° C. for 8 hours, while in the second experiment the digestions were continued for only 3½ hours, yet in the latter case a very great increase in digestive action is to be noted; an increase which is to be attributed mainly to the greater concentration of the papoid solution. This constitutes a very good illustration of what I have found to be characteristic of papoid digestion in general, and with all forms of proteid matter. Papoid will act in dilute solutions, but the best and characteristic action is seen only when a small volume of fluid is present. In this respect, it differs very markedly from the animal ferment pepsin, and for this reason any direct comparison of the two ferments is practically impossible, since they act best under such widely different conditions.

It is well-known that raw blood-fibrin, like raw beef tissue, is more or less attacked by dilute acids and alkalies alone, especially at the body-temperature, but blank experiments, without papoid, show that these reagents have in themselves only a comparatively slight solvent action on raw fibrin; thus, in one experiment where 10 grams of moist, raw blood-fibrin were exposed to the action of 25 c. c. of an 8 per cent. solution of sodium bicarbonate at 45° C. for 6 hours, only 5·2 per cent. of the dry proteid was dissolved.

e. Action on Boiled Blood-fibrin.

The fibrin was simply well washed blood-fibrin, boiled with water until it was thoroughly coagulated, and then pressed as dry as possible.

Each digestive mixture contained 0·5 gram papoid, 10 grams of the prepared fibrin, and 25 c. c. of water with the respective percentages of acid and alkali.

The 10 grams of boiled fibrin contained 3·692 grams of dry proteid (dried at 110° C.).

The mixtures were warmed at 45° C. for 9 hours.

Reaction.	Weight of undigested residue.	Boiled fibrin digested.
Neutral	2·6397 grams	28·5 per cent.
0·05 per cent. Hydrochloric acid	2·5810 "	30·0 "
0·10 " " "	2·7399 "	25·7 "
2·00 " Bicarb. soda	2·5875 "	29·9 "

From these results, it is evident that there is a far greater difference in digestibility between raw and cooked fibrin, than between raw and cooked beef proteids. With the latter proteids, there is no very pronounced difference in digestibility whether they are cooked or raw, but with blood-fibrin the difference is very great. Boiled fibrin appears to be quite resistant to the action of papoid, while raw fibrin, on the other hand, is extremely susceptible to the action of the ferment. By long continued digestion, however, boiled fibrin gradually succumbs, and eventually can be almost completely digested.

Casting a backward glance over the foregoing results, it is evident that the statement made at the outset, that papoid has the power of digesting all common forms of proteid matter, both in a neutral, acid and alkaline medium, is well substantiated by the facts. Papoid is peculiar in that it will digest and dissolve proteid matter in a neutral solution, in this respect resembling trypsin, the proteolytic ferment of the pancreatic juice. But trypsin is a ferment associated with an alkaline secretion, and as a proteolytic agent acts to advantage only in alkaline fluids. Papoid agrees with trypsin in so far that its proteolytic action is increased by the presence of an alkaline medium, in some cases greatly increased by the presence of 2–4 per cent. sodium bicarbonate. On the other hand, the action of papoid in a neutral solution is increased by the addition of very small amounts of hydrochloric acid, and in some cases the increase is very marked. Just here, attention should be called to a statement previously made, viz: that an aqueous solution of papoid is not absolutely neutral, but shows, on careful testing, a very faint trace of alkalinity. Consequently, a minute portion of the acid added, may be taken up in bringing about a more complete neutralization of the mixture, although the amount must be too small to have much influence on the final result.

Another action to be noted as characteristic of papoid digestion is the peculiar physical change it produces in the proteid acted upon. The exact character of this change is dependent upon the condition of the proteid and, in part, upon the character of the medium in which the digestion is carried on. Thus, with a coagulated proteid,

as cooked beef, there is a rapid disintegration and falling apart of the proteid into tiny fragments, until at last the undigested matter has the character of a pultaceous residue. Hence, in this respect papoid resembles trypsin. This peculiarity is especially noticeable in the digestion of cooked beef, or boiled fibrin, with papoid in the presence of 0·1 per cent., and even 0·2 per cent., hydrochloric acid. The natural tendency of the acid under such circumstances, especially at 45° C., is to cause the beef fibres to swell up, but this tendency is gradually counteracted by the presence of papoid, and eventually, but more slowly, there comes about the same disintegration of the proteid seen in the neutral or alkaline solution. With a raw or non-coagulated proteid, on the other hand, there is at first, especially in an alkaline solution (sodium bicarbonate), a softening action, which is in great part independent of the alkali; followed, it may be, by the formation of an almost solid, homogeneous, jelly-like mass, in which the fibres lose their individuality, thus differing from the swelling produced by the alkaline fluid alone. As digestion proceeds, the jelly-like mass gradually becomes thinner from the secondary or solvent action of the ferment. The same softening, though less pronounced, shows itself with a neutral solution of papoid, followed by more or less disintegration, although the residue never takes on the appearance seen in the digestion of a cooked proteid.

3. *Circumstances modifying the proteolytic action of papoid.*

Under this head we have to consider more especially: first, the influence of reaction and temperature on the proteid-digesting power of papoid; and secondly, the influence of such drugs or therapeutic agents as would most naturally be combined with papoid in medical practice.

a. Influence of Reaction.

As regards the influence of the reaction of papoid solutions upon the activity of the ferment, we have already demonstrated that with coagulated proteids the highest digestive power is obtained in the presence of sodium bicarbonate, 2–4 per cent. Furthermore, that while the ferment is extremely active in a neutral solution, its activity is, as a rule, increased slightly by the addition of small amounts of very dilute hydrochloric acid, which increase becomes very marked in the case of raw or non-coagulated proteids. With coagulated proteids, the increased digestive action due to the presence of a small amount of very dilute hydrochloric acid is not

anywhere near equal to the increase produced by the presence of sodium bicarbonate. Hydrochloric acid much above 0·2 per cent. inhibits almost entirely the proteolytic action of the ferment.

Obviously, however, we cannot consistently argue from these facts that any acid-reacting fluid will produce the same result as hydrochloric acid, or that any alkaline-reacting fluid will give the same marked increase in digestive action as sodium bicarbonate. These points are well illustrated by the following results, which at the same time show the influence of several common substances upon papoid digestion of various proteids.

Influence of Sodium Carbonate on Papoid Digestion of Cooked Beef.

In this, as in the following series of experiments, unless otherwise specified, each digestive mixture contained 0·5 gram papoid, 10 grams of the proteid designated (in this case cooked beef proteids, prepared as previously described), and 25 c. c. of water containing the indicated percentages of sodium carbonate, or other substances.

The 10 grams of cooked beef contained 3·7767 grams of dry proteid (110° C.).

The digestions were warmed at 45° C. for 5½ hours.

Sodium carbonate.	Weight of undigested residue.	Proteid digested.
0 (Neutral)	1·8424 grams	51·2 per cent.
0·25 per cent.	1·7697 "	53·1 "
0·50 "	1·7906 "	52·5 "
1·00 "	1·8934 "	49·8 "
2·00 "	1·8541 "	50·9 "
4·00 "	2·1562 "	42·9 "
Without papoid.		
2·00 per cent.	3·4607 "	8·3 "

These results show that the presence of sodium carbonate in small quantity tends to increase slightly the digestive action of papoid. Aside from this slight increase, the activity of the ferment is not noticeably affected by the alkaline salt until it is present in quantities above 2 per cent., and even then the inhibition is comparatively slight. This seems the more remarkable when it is remembered that sodium carbonate is a fairly strong alkaline salt, and doubtless on that very account fails to produce the marked increase in digestive action produced by the weaker bicarbonate. In any event, it is evident, from the experiment, that papoid will not be

checked in its digestive action by contact with the alkaline fluids of the intestinal tract.

Another very noticeable action of papoid was seen in connection with this experiment. Sodium carbonate, especially the larger percentages, tends to produce a very noticeable and pronounced swelling of the coagulated proteid. Thus, in the control experiment without papoid, where 10 grams of the cooked beef proteids were warmed at 45° C. with 2·0 per cent. sodium carbonate, the proteid matter was quickly swollen to an almost solid jelly, but in the presence of papoid this swelling of the proteid was wholly absent, even when the alkali carbonate was increased to 4·0 per cent; in the presence of papoid, the undigested residue was thoroughly disintegrated and pultaceous. Papoid thus counteracts the swelling action of the alkaline fluid in the same manner as it counteracts the swelling action of dilute hydrochloric acid, already referred to.

Influence of Boracic Acid on Papoid Digestion.

a. With raw beef proteids,—10 grams contained 2·8508 grams of dry proteid (110° C.). The digestions were warmed at 45° C. for 7 hours.

Boracic Acid.	Weight of undigested residue.	Proteid digested.
0 (Neutral)	0·8988 gram	68·4 per cent.
2·0 per cent.	0·8210 "	71·2 "

b. With cooked beef proteids,—10 grams contained 3·7438 grams of dry proteid (110° C.). Digestions were warmed at 45° C. for 5½ hours.

Boracic Acid.	Weight of undigested residue.	Proteid digested.
0 (Neutral)	1·7782 grams	52·5 per cent.
2·0 per cent.	1·5950 "	57·3 "

c. With boiled blood-fibrin,—10 grams contained 3·692 grams of dry proteid (110° C.). Digestions were warmed at 45° C. for 9 hours.

Boracic Acid.	Weight of undigested residue.	Proteid digested.
0 (Neutral)	2·6397 grams	28·5 per cent.
2·0 per cent.	2·7705 "	24·9 "

d. With coagulated egg-albumin,—10 grams moist coagulum contained 1·5143 grams of dry proteid (110° C.). Digestions were warmed at 45° C. for 12 hours.

Boracic Acid.	Weight of undigested residue.	Proteid digested.
0 (Neutral)	0·6503 gram	57·0 per cent.
2·0 per cent.	0·6012 "	60·2 "

With the exception of the single experiment with boiled blood-fibrin, all of the results show that the proteid-digesting power of papoid is distinctly increased by the presence of boracic acid, at least by the percentages of acid indicated. Consequently, it is plain that the combination of boracic acid with papoid will not inhibit the action of the ferment, but will, on the contrary, increase its proteid-dissolving power.

Influence of Acetic and Lactic Acids on Papoid Digestion of Raw Beef Proteids.

The 10 grams of moist, prepared beef contained 2·8508 grams of dry proteid (110° C.). The digestions were warmed at 45° C. for 6½ hours.

Reaction.		Weight of undigested residue.	Proteid digested.
Neutral		0·9538 gram	66·5 per cent.
0·5 per cent.	Acetic acid	1·6115 "	43·4 "
1·0 "	"	1·5976 "	43·9 "
0·5 "	Lactic acid	1·3796 "	51·6 "
1·0 "	"	1·6823 "	40·9 "

The percentages of the two acids used in this experiment are somewhat high, considering the strength of the acids, but the object in view was especially to ascertain the probable effect on the ferment of admixture with an organic acid, such as might be developed in large quantity in the stomach in a case of strong acidity from lactic or butyric acid fermentation. The results show that the ferment is inhibited somewhat in its digestive action by the presence of these large percentages, although not to any very great extent. Possibly, the addition of smaller quantities of these acids might lead to an increase in digestive power over that of the neutral solution.

Influence of Lime Water on Papoid Digestion of Raw Beef Proteids.

The 10 grams of prepared beef contained 2·707 grams of dry proteid (110° C.). The mixtures were warmed at 45° C. for 6½ hours.

Medium.	Weight of undigested residue.	Proteid Digested.
25 c. c. Water	0·7175 gram	73·4 per cent.
12½ c. c. Water 12½ " Lime water*	0·8076 "	70·1 "

Here, the percentage of lime water was quite large, but the object in view was to ascertain quickly whether, under any ordi-

* A saturated aqueous solution of calcium hydroxide.

nary circumstances, as in the peptonization of milk, the presence of this weak alkali would offer any hindrance to the digestive action of papoid. The results show that when lime water is present to the extent of even 50 per cent. of the total volume, digestion is not materially interfered with. Indeed, in another experiment, where papoid was dissolved directly in 25 c. c. of lime water and no other fluid added, 53·7 per cent. of the raw beef proteid was converted into soluble products, while in the control digestion (papoid in water) 66·5 per cent. of the proteid matter was dissolved; thus showing that lime water, even when present in large amount, has little power to hinder the proteolytic action of papoid.

b. Influence of Temperature.

Animal ferments, especially those concerned in the ordinary processes of digestion, act most energetically, as is well-known, at approximately the body temperature. Raising the temperature to near 60° C. quickly brings about a diminution in digestive action, followed by a gradual destruction of the ferment. Lowering the temperature below that of the body is likewise accompanied by a diminution in digestive action, although ordinarily less marked than the inhibition caused by a rise of temperature. With the animal proteolytic ferments, pepsin and trypsin, digestion is very slow at, say 20° C. With diastase, the vegetable amylolytic ferment, the most rapid conversion of starch into sugar takes place at about 55° C.

A study of papoid digestion, with reference to this point, has revealed a very interesting peculiarity of this ferment, viz: a great resistance towards inhibition of digestive action by high temperatures. Thus, in an acid solution (boracic acid), a larger amount of cooked beef proteids is dissolved at 70° C. than at any lower temperature, while even boiling the ferment solution fails to destroy entirely the action of the ferment; a fact which is especially true of an alkaline (sodium bicarbonate) solution of papoid. Furthermore, at a comparatively low temperature, 20° C., digestion is very pronounced in both a neutral and acid solution of the ferment, while in an alkaline fluid, digestive action is almost as great at 20° C. as at 45° C.

The three following series of experiments, illustrative of these points, were made at the same time and under exactly the same conditions, excepting the specified variations in temperature and reaction. Each digestive mixture contained 0·5 gram papoid, 10

grams of prepared cooked beef and 25 c. c. of fluid; the latter consisting of water alone in the neutral series, of 2·0 per cent. boracic acid-solution in the acid series, and of 2·0 per cent. sodium bicarbonate-solution in the alkaline series.

The digestions were continued at the given temperatures for 6 hours, the fluid itself being brought to the required temperature before the proteid was added.

The 10 grams of cooked beef contained 3·5538 grams of dry proteid (dried at 110° C.).

NEUTRAL SOLUTION.

Temperature.	Weight of undigested residue.	Proteid digested.
20° C.	2·2445 grams	36·8 per cent.
40	1·4743 "	58·5 "
45	1·5640 "	55·9 "
60	1·1773 "	66·8 "
70	1·1975 "	66·3 "
Solution first boiled and then kept at 45° C.	3·2286 "	9·1 "

ALKALINE SOLUTION.

Temperature.	Weight of undigested residue.	Proteid digested.
20° C.	1·4776 grams	58·4 per cent.
40	1·2796 "	63·9 "
45	1·3054 "	63·2 "
60	1·0080 "	71·6 "
70	1·1081 "	68·8 "
Solution first boiled and then kept at 45° C.	2·4708 "	30·4 "

ACID SOLUTION.

Temperature.	Weight of undigested residue.	Proteid digested.
20° C.	2·2153 grams	37·6 per cent.
40	1·4695 "	58·6 "
45	1·5240 "	57·1 "
60	1·2184 "	65·7 "
70	1·0804 "	69·5 "
Solution first boiled and then kept at 45° C.	3·1560 "	11·1 "

The results brought out by these three series of experiments can be more readily seen by a direct comparison of the percentages digested under the different conditions, as shown in the following table :

Temperature.	Proteid digested. Neutral sol.	Acid sol.	Alkaline sol.
20° C.	36·8 per cent.	37·6 per cent.	58·4 per cent.
40	58·5	58·6	63·9
45	55·9	57·1	63·2
60	66·8	65·7	71·6
70	66·3	69·5	68·8
Solution first boiled and then kept at 45° C.	9·1	11·1	30·4

We notice first in these results a repetition of what has been already demonstrated, viz: that in an acid solution (boracic acid), papoid is slightly more active than in a neutral fluid, while in an alkaline solution (sodium bicarbonate) the activity of the ferment is increased very greatly. We now see that this statement holds good practically for all temperatures. The most striking facts, however, brought out by these experiments are: first, the marked activity of the ferment at the comparatively low temperature of 20° C. (the temperature of the room at the time the experiment was tried), especially in an alkaline fluid; and secondly, the retention of proteolytic power after the solution of the ferment has been actually boiled. Here, too, the alkaline solution appears to exert a certain protective influence upon the ferment, which is difficult to explain. Certainly, sodium bicarbonate alone will not dissolve a coagulated proteid to any great extent, as has been already demonstrated in connection with other experiments. Hence, we are forced to the conclusion that in an alkaline fluid especially, papoid is extremely resistant to the inhibitory effects of low and high temperatures, so characteristic of most known ferments. This being true, it is obvious that papoid in the presence of sodium bicarbonate possesses special advantages in cases where it is desired to soften or digest tissue or other proteid matters, at comparatively low temperatures. In view of the importance of this fact, a duplicate experiment was tried, in which the digestive action of papoid was again tested at the room temperature (21–22° C.) on cooked beef proteids, in a neutral solution, and in the presence of boracic acid and sodium bicarbonate. The 10 grams of prepared beef contained 3·7550 grams of dry proteid (dried at 110° C.). The digestions were continued for 6 hours, at the given temperature.

Reaction.	Weight of undigested residue.	Proteid digested.
Neutral	2·4053 grams	35·9 per cent.
2·0 per cent. Boracic acid	2·2394 "	40·3 "
2·0 " Bicarb. soda	1·9311 "	48·5 "
Without papoid.		
2·0 per cent. Bicarb. soda	3·6161 "	3·6 "

Here, the same results are to be seen as in the preceding experiment, although the difference between the acid and the alkaline digestion is not quite as pronounced. Still, the results certainly warrant the conclusion already advanced, that in papoid we have a proteolytic agent especially adapted for the digestion of proteid matter at comparatively low temperatures. At the same time, it is a ferment very resistant to the ordinary destructive effects of high temperatures, and is especially characterized by exhibiting its maximum digestive power at about 70° C.

c. Influence of various Therapeutic Agents and other Substances on the Proteolytic Action of Papoid.

In this connection, those substances have been chosen which might naturally be combined with papoid in its application as a therapeutic agent, either internally or externally, or which might perchance exert some modifying influence upon the action of the ferment as a general proteolytic agent. The experiments have been conducted in the same manner as those already described; each digestive mixture containing 0·5 gram papoid, 10 grams of prepared beef proteids, raw or cooked, and 25 c. c. of water, together with the specified percentage of the substance to be tested; the digestive action in each case being compared with that of a control digestion composed of papoid, proteid, and water alone.

Salicylic Acid.

The mixtures were warmed at 45° C. for 8 hours.

The 10 grams of raw beef proteids contained 2·743 grams of dry proteid (110° C.).

Salicylic acid.	Weight of undigested residue.	Proteid digested.
0 (neutral)	1·1070 grams	59·6 per cent.
0·1 per cent.	0·9536 "	65·2 "
0·2 "	0·9233 "	66·3 "

From these results, it is evident that salicylic acid in small quantities tends to increase the proteolytic action of papoid over that of a neutral solution. As is well known, a 0·2 per cent. solution of salicylic acid is amply strong to act as an efficient antiseptic, preventing the appearance of putrefaction in an organic fluid, even under the most favorable circumstances for its development. Consequently, salicylic acid and papoid might well be combined where application of the ferment to morbid or suppurating growths is desired.

Carbolic Acid.

The mixtures were warmed at 45° C. for 6½ hours.

The 10 grams of raw beef contained 2·8508 grams of dry proteid (110° C.).

Carbolic acid.	Weight of undigested residue.	Proteid digested.
0 (neutral)	0·9538 gram	66·5 per cent.
0·5 per cent.	1·1441 "	59·8 "
1·0 "	1·2270 "	56·9 "

Hence, carbolic acid inhibits slightly the proteolytic action of papoid, but not to any great extent; the ferment will still act vigorously, even in the presence of 1·0 per cent. of the acid.

Mercuric Chloride.

The 10 grams of prepared raw beef contained 2·6996 grams of dry proteid (dried at 110° C.).

The mixtures were warmed at 45° C. for 6½ hours.

Mercuric Chloride.	Weight of undigested residue.	Proteid digested.
0	0·9758 gram	63·8 per cent.
0·05 per cent.	1·0660 "	60·5 "

In another similar experiment, but with 0·1 per cent. mercuric chloride, 57·3 per cent. of the proteid matter was dissolved, while in the control digestion 59·6 per cent. was converted into soluble products. Hence, mercuric chloride or corrosive sublimate, when present in a neutral solution of papoid to the extent of 0·1 per cent., does not materially interfere with the proteolytic action of the ferment. This seems somewhat remarkable, and in conjunction with the two preceding experiments makes clear that papoid, as a proteolytic agent, is not checked to any extent in its digestive action by three of the best known antiseptics.

Chloroform.

The 10 grams of prepared raw beef contained 2·713 grams of dry proteid (110° C.).

The mixtures were warmed at 45° C. for 8 hours.

Chloroform.	Weight of undigested residue.	Proteid digested.
0	1·1070 grams	59·6 per cent.
4·0 per cent.	1·2013 "	56·2 "
0	0·9758 gram	63·8 "
8·0 per cent.	1·3423 "	50·2 "

In the second experiment, with 8 per cent. of chloroform, the two mixtures were warmed at 45° C. for 7 hours, and the 10 grams of

raw beef proteids contained 2·6996 grams of dry proteid. Obviously, in these experiments a portion of the chloroform evaporates during the seven or eight hours, but the greater portion remains to exert such influence as it possesses. The results show some inhibition of ferment action, but it is not very pronounced unless the amount of chloroform is raised to more than 4 per cent.

Thymol.

The 10 grams of prepared raw beef proteids contained 2·8508 grams of dry proteid (110° C.).

The two mixtures were warmed at 45° C. for 7 hours.

Medium.	Weight of undigested residue.	Proteid digested.
Water	0·8988 gram	68·4 per cent.
Water and thymol	1·0250 "	64·0 "

A few drops of thymol solution (20 per cent. thymol in alcohol) were added to the one digestive mixture from time to time, in such quantity that there was always a strong odor of thymol, and a thin film of the substance floating on the top of the fluid. As is well known, thymol is widely used in artificial digestion experiments, to prevent possible putrefaction, as it, like chloroform, ordinarily exerts only a minimal interference with the action of the unorganized ferments or enzymes. The above result shows that thymol has only the same slight inhibitory effect on papoid digestion.

Hydrogen Peroxide.

There is no very satisfactory way of measuring the exact influence of hydrogen peroxide on the digestive action of papoid, or any other like ferment. The only way available was to dissolve the 0·5 gram of papoid in 25 c. c. of the peroxide solution (Marchand's), and then to add the 10 grams of prepared raw beef. Naturally, the peroxide solution produced an immediate and pronounced change in the character of the proteid, and doubtless such diminution of digestive action as is apparent from the result is attributable to an alteration in the proteid, rather than to any change in the ferment itself. The experiment may therefore well be taken as illustrative of the extent to which a comparatively large amount of hydrogen peroxide will convert the easily digestible proteids of raw tissue into more difficultly digestible products. A control experiment, with 25 c. c. of the peroxide solution alone, shows that a small amount of the raw proteid is dissolved by this agent itself.

The 10 grams of prepared raw beef contained 2·7071 grams of dry proteid (dried at 110° C.).

The mixtures were kept at 40–45° C. for 7 hours.

Medium.	Weight of undissolved residue.	Proteid dissolved.
Papoid & water	0·9828 gram	63·6 per cent.
Papoid & hydrogen peroxide.	1·3812 "	48·9 "
Hydrogen peroxide alone.	2·5051 "	7·4 "

With cooked beef proteids, hydrogen peroxide so alters the character of the material, that papoid cannot exert any solvent action whatever upon it, and the peroxide solution alone is not able to dissolve any of the altered proteid.

Potassium Chlorate.

The 10 grams of raw beef proteids contained 2·707 grams of dry proteid (110° C.).

The mixtures were warmed at 45° C. for 6½ hours.

Potass. Chlorate.	Weight of undigested residue.	Proteid digested.
0	0·7175 gram	73·4 per cent.
1·0 per cent.	0·8245 "	69·5 "
2·0 "	0·7948 "	70·6 "

Here, we see a very slight inhibitory action, so slight as to have little significance. Practically, potassium chlorate is without any marked hindering action on papoid digestion.

Sodium Chloride.

With this salt, three distinct series of experiments were tried, in order to ascertain its influence on the activity of the ferment in neutral, acid and alkaline fluids. The proteid material employed was prepared cooked beef, 10 grams of which contained 3·7815 grams of dry proteid (dried at 110° C.).

All of the mixtures were warmed at 45° C. for 5½ hours.

a. In neutral solution.

Sodium chloride.	Weight of undigested residue.	Proteid digested.
0	1·9017 grams	49·7 per cent.
1·0 per cent.	1·8091 "	52·1 "
2·0 "	1·7596 "	53·4 "
4·0 "	1·7853 "	52·7 "

b. In alkaline solution, 2·0 per cent. sodium bicarbonate.

Sodium chloride.	Weight of undigested residue.	Proteid digested.
0	1·6233 grams	57·7 per cent.
1·0 per cent.	1·6379 "	56·6 "
2·0 "	1·6779 "	55·6 "
4·0 "	1·7450 "	53·8 "

c. In acid solution, 0·1 per cent. hydrochloric acid.

Sodium chloride.	Weight of undigested residue.	Proteid digested.
0	2·7567 grams	27·1 per cent.
1·0 per cent.	2·2862 "	39·5 "
2·0 "	2·2780 "	39·7 "
4·0 "	2·3344 "	38·2 "

d. In acid solution, 0·2 per cent. hydrochloric acid.

The 10 grams cooked beef proteids contained 3·7767 grams of dry proteid (110° C.).

Sodium chloride.	Weight of undigested residue.	Proteid digested.
0	3·2872 grams	12·9 per cent.
1·0 per cent.	3·1226 "	17·3 "
2·0 "	3·1138 "	17·5 "
4·0 "	3·1023 "	17·9 "

Percentages of Proteid Digested.

Sodium chloride.	Neutral solution.	2·0 p. c. Bicarb. soda.	0·1 per cent. HCl.	0·2 per cent. HCl.
0	49·7 per cent.	57·7 per cent.	27·1 per cent.	12·9 per cent.
1·0 per cent.	52·1	56·6	39 5	17·3
2·0	53·4	55·6	39·7	17·5
4·0	52·7	53·8	38·2	17·9

From these results, it is plain that sodium chloride or common salt increases slightly the solvent action of papoid on coagulated proteids in neutral solutions; while in an acid solution, 0·1 per cent. and 0·2 per cent. hydrochloric acid, it increases very greatly the solvent power of the ferment. This action of the salt we shall see later is connected with a certain solvent power on one or more of the products of digestion, especially formed in acid solutions of papoid. Salt by itself, or in connection with dilute acid, has practically no power of dissolving the proteids of coagulated beef. In fact, the presence of salt diminishes decidedly the ordinary solvent action exerted by dilute acid alone. Thus, 0·2 per cent. hydrochloric acid by itself, at 45° C., will dissolve about 4·6 per cent. of proteid matter from the 10 grams of coagulated beef in 5½ hours; while in the presence of 2·0 per cent. of sodium chloride, acid of the same strength, under like conditions, will dissolve only 2·6 per cent. of the proteid. The salt counteracts the swelling action of the dilute acid and thus diminishes its direct solvent power.

In an alkaline solution of papoid, salt appears to inhibit very slightly the proteolytic action of the ferment.

Bismuth Sub-nitrate.

The 10 grams of prepared raw beef contained 2·707 grams of dry proteid (110° C.).

The mixtures were warmed at 45° C. for 7 hours.

Bismuth sub-nitrate.	Weight of undigested residue.	Proteid digested.
0 (Neutral)	0·9043 gram	66·5 per cent.
1·0 per cent.	1·4816 "	45·2 "
2·0 "	1·6324 "	39·6 "

This salt, as is evident from the results, diminishes quite decidedly the proteolytic action of the ferment, although digestion will still go on even when the salt is present in large excess.

Bismuth Ammonium Citrate (soluble).

The 10 grams of raw beef contained 2·2628 grams of dry proteid (110° C.).

The mixtures were warmed at 45° C. for 8 hours.

Bismuth salt.	Weight of undigested residue.	Proteid digested.
0 (neutral).	0·5468 gram	75·8 per cent.
1·0 per cent.	1·2583 "	44·3 "
2·0 "	1·2164 "	46·2 "
4·0 "	1·0027 "	55·6 "

Here, as with bismuth sub-nitrate, we see a decided falling off in digestive power in the presence of the bismuth salt. The apparent slight increase in digestive power coincident with the increase in the percentage of the salt is probably due to the somewhat greater alkalinity, which presumably counteracts, in part, the inhibitory action of the bismuth portion of the salt.

Peppermint Oil.

The 10 grams of raw beef proteids contained 2·6996 grams of dry proteid (110° C.).

The mixtures were warmed at 45° C. for 6½ hours.

Peppermint oil.	Weight of undigested residue.	Proteid digested.
0 (Neutral)	0·9758 gram	63·8 per cent.
4·0 per cent.	1·0433 "	61·3 "

Obviously, the above percentage of oil was not wholly dissolved, but it insured a fairly large excess present throughout the experi-

ment. This, as seen from the result, did not materially interfere with the digestive action of the ferment.

Strychnin and Brucin Sulphates.

The 10 grams of prepared raw beef contained 2·707 grams of dry proteid (dried at 110° C.).

The mixtures were warmed at 45° C. for 6½ hours.

Alkaloid salt.	Weight of undigested residue.	Proteid digested.
0	0·7175 gram	73·4 per cent.
0·25 per cent. Strych. sulph.	1·2006 "	55·6 "
0·50 " "	0·9089 "	66·4 "
0·25 " Brucin sulph.	0 8461 "	68·7 "
0·50 " "	0·8068 "	70·1 "

These results show that the two alkaloidal salts have a tendency to diminish the digestive power of papoid; a tendency which is more pronounced with strychnin than with brucin, the inhibitory action in the latter case being comparatively slight. Doubtless, the increase in digestive action coincident with the increase in the percentage of the alkaloids is due to the slight acidity of the salts, which presumably overcomes in part the retarding effect of the alkaloidal base.

Antipyrin and Acetanilid.

The 10 grams of prepared raw beef contained 2·6996 grams of dry proteid (110° C.).

The mixtures were warmed at 45° C. for 6½ hours.

Medium.	Weight of undigested residue.	Proteid digested.
Control	0·9758 gram	63·8 per cent.
1·0 per cent. Antipyrin	0·9500 "	64·8 "
2·0 " "	1·0803 "	59·9 "
4·0 " "	1·2992 "	51·8 "
1·0 " Acetanilid	0·9102 "	66·2 "
2·0 " "	1·0497 "	61·1 "

These two drugs agree in producing a very slight increase in proteolytic action when present in the digestive mixture in moderate amounts, followed in the case of antipyrin by a decreased digestive action when the amount present reaches 4·0 per cent.

Quinine Sulphate.

With this salt, there is, as the results show, a slight inhibitory action on the digestive power of the ferment, but not sufficient to materially interfere with its proteolytic action.

The 10 grams of raw beef used in this experiment contained 2·707 grams of dry proteid (110° C.).

The mixtures were warmed at 45° C. for 7 hours.

Quinine sulphate.	Weight of undigested residue.	Proteid digested.
0	0·9043 gram	66·5 per cent.
1·0 per cent.	1·1832 "	56·2 "
2·0 "	1·0026 "	62·9 "

In view of the results obtained in the majority of the preceding experiments, it would seem that papoid is characterized by a fair degree of resistance towards the usual inhibitory action of many common therapeutic agents. Certainly, the foregoing results show that papoid is able to exert its ordinary proteid-digesting power under many diverse conditions, a fact which gives it added value as a therapeutic agent.

4.—*The products of papoid digestion.*

The foregoing experiments, taken collectively, testify to the proteid-dissolving power of papoid under a great diversity of conditions. They do not, however, show that this solvent power is necessarily akin to, or identical with, that of the ordinary digestive ferments. As is well known, the latter agents exert their solvent action by virtue of certain chemical changes they induce, as a result of which new and for the most part soluble products result, of which the proteoses, or albumoses, and peptones are the principal representatives. It is to be presumed, however, that papoid acts in a similar manner. Indeed, Martin* long ago pointed out that the proteolytic ferment of papaw juice, acting on blood-fibrin, formed large quantities of peptone, together with leucin and tyrosin, as products of its digestive action. Such experiments as I have tried bearing on this point clearly show that the proteid-dissolving power of papoid is due to a genuine ferment action, whereby soluble products are formed which, so far as ordinary chemical reactions will show, are closely akin to, or identical with, those formed in gastric and pancreatic digestion. Leucin and tyrosin are likewise formed, thus showing in another way the resemblance of this ferment to the trypsin of the pancreatic juice.

While, in a general way, the final products of papoid digestion are essentially the same under all ordinary circumstances, certain minor differences appear in the primary or side-products, coincident with

* Journal of Physiology, vol. v, p. 225.

changes in the reaction of the digestive fluid, and in the nature of the proteid undergoing digestion. Some of these points may be briefly summarized.

In the digestion of coagulated egg-albumin with an alkaline (2·0 per cent. sodium bicarbonate) solution of papoid, even when the digestion has been long continued (say 18 hours) at a favorable temperature, and the ferment solution strong, there invariably remains a fairly large undissolved residue. At first glance, this would naturally appear to be simply a residue of unaltered, coagulated albumin. On being tested, however, it is found soluble, at least in great part, in warm 0·2 per cent. hydrochloric acid, from which solution it is reprecipitated by addition of 0·5 per cent. sodium carbonate and redissolved by an excess of the alkaline fluid. This residue is, likewise, directly soluble in warm 0·5 per cent. sodium carbonate, and reprecipitated by neutralization. These two reactions clearly indicate that the above residue cannot be composed of unaltered coagulated albumin, since this substance is wholly insoluble in dilute acid and alkali. The only plausible inference, therefore, is that the so-called undigested residue in this case is composed of an albumose-like body insoluble in 2·0 per cent. sodium bicarbonate, a possible primary or side-product of the papoid digestion of coagulated egg-albumin. It is evidently a somewhat unique body, differing from hetero-albumose, and from ordinary globulin, by being insoluble in salt solution. Aside from this peculiar insoluble body, the other products of digestion isolated in the single experiment tried were a deutero-albumose, a fairly large amount of peptone and some leucin and tyrosin. Only a trace of protoalbumose was found, and no hetero. Neutralization of the clear, alkaline digestive fluid failed to give any precipitate, as did also boiling the neutralized solution. In fact, all of the ordinary primary products of digestion seemed in this case to be replaced by the above described insoluble albumose, composing the so-called undigested residue. Of the soluble products, deuteroalbumose and peptone predominated.

In the digestion of raw blood-fibrin with a neutral solution of papoid, a somewhat different condition of things was observed. The undissolved residue contained, perhaps, a small amount of the body so characteristic of the digestion of coagulated egg-albumin, but certainly not a large amount. The clear, filtered digestive fluid, however, gave evidence of the presence of a peculiar body which was wholly wanting in the digestion of the coagulated albumin. Thus, the addition of water to the clear neutral fluid gave

a heavy white precipitate, of what was later proved to be an albumose-like body, readily soluble in a little 10 per cent. salt solution. Addition of 0·2 per cent. hydrochloric acid, likewise, produced a heavy precipitate of the same body, easily soluble in a slight excess of the acid. Boiling the neutral solution also gave rise to a heavy precipitate or coagulum, apparently the same body as that precipitated by water and by dilute acid, slowly but completely soluble in warm 0·2 per cent. hydrochloric acid, and in warm 0·5 per cent. sodium carbonate-solution. Hence, this body is a soluble albumose and not a coagulable globulin. It is completely precipitable from a neutral solution by heat, and partakes of the general character of heteroalbumose, being insoluble in water but completely soluble in salt solutions, as well as in dilute acid and alkali. In addition to this peculiar primary product of digestion, there was also found a large amount of more soluble primary and secondary albumoses, together with true peptone, leucin and tyrosin.

Raw blood-fibrin, digested with a weak hydrochloric acid (0·05 per cent.) solution of papoid, yields the same products as those just described; the peculiar primary albumose making its appearance here in fully as large quantity as in the neutral digestion and apparently taking the place of acid-albumin, which in the single experiment tried appeared to be entirely wanting.

In the digestion of cooked beef proteids with a neutral solution of papoid, as likewise with an alkaline solution of the ferment, the peculiar heteroalbumose-like body above described was wholly absent; only the ordinary primary and secondary albumoses were observed, together with a large amount of peptone and some leucin and tyrosin.

The above results, therefore, plainly warrant the statement that the power possessed by papoid of dissolving various forms of proteid matter is dependent upon an ordinary digestive action akin to, or identical with, that of digestive ferments in general, whether animal or vegetable.

5.—*Action of Papoid on Milk.*

The action of papoid on milk is twofold. First, under suitable conditions, it brings about a curdling of the milk or separation of the casein, more or less complete according to the circumstances. This is followed by the ordinary digestive action of the proteolytic ferment, in which the precipitated casein is gradually converted, wholly or in part, into soluble products.

The act of curdling, like the process of digestion, is modified more or less by the conditions under which the experiment is tried. Thus, under some circumstances the curdling takes place quickly and the separation of the casein is quite complete. Under others, the curdling takes place slowly and is very incomplete. These points are well illustrated by the following experiments: Each mixture had a total volume of 100 c. c., composed of 25 c. c. of milk, either fresh or boiled as indicated, 25 c. c. of an aqueous solution of papoid (0·5 gram papoid), and 50 c. c. of water containing sodium bicarbonate as indicated, or else an equal volume of lime water and water as specified. Some of the mixtures were kept at a temperature of 40–45° C., while others were allowed to stand at the room temperature, viz: 22–24° C. Following, are the results obtained under the different conditions:

At 40–45° C.

Character of the milk.	Reaction of the mixture.	Time of curdling.
Boiled	Neutral	3 minutes
Fresh	"	10 "
"	10 per cent. lime water	11 "
"	20 " "	12 "
"	2·0 " Bicarb. soda*	19 "
"	1·0 " "	35 "
"	0·5 " "	55 "
Boiled	2·0 " "	240 "

In the presence of 2·0 per cent. sodium bicarbonate, the boiled milk was very incompletely curdled; apparently, the digestion of the casein was quite advanced before any sign of separation could be observed. In the neutral solution, on the other hand, the curdling of the boiled milk took place almost immediately, as noted, and was at the same time very complete, the casein separating as a fine flocky precipitate, leaving an almost clear fluid. On longer standing at 40° C., the separated casein was, however, gradually dissolved. In the presence of 0·5 and 1·0 per cent. sodium bicarbonate, the curdling of the fresh milk was not as complete as when 2·0 per cent. of the bicarbonate was present. This would naturally be expected, since the longer the curdling is delayed the less unaltered casein will there be to separate. In all of the above cases where the curdling took place inside of 20 minutes the separation of the casein was fairly complete.

* The percentages refer to the total amount of bicarbonate, or other substance, contained in the 100 c. c. of fluid.

At 22–24° C.

Character of the milk.	Reaction of the mixture.	Time of curdling.
Boiled	Neutral	12 minutes.
Fresh	"	145 "
"	20 per cent. lime water	Not curdled at the end of 4 hours.
Boiled	" "	
"	2·0 " Bicarb. soda	
Fresh	" "	

It is thus evident, from the above experiments, that the curdling of milk by papoid is greatly modified by the temperature of the fluid. It is not to be assumed, however, that while precipitation of the casein is delayed by a low temperature, digestion is equally retarded. Such is certainly not the case, for digestion of the casein, whether still in solution or precipitated by the curdling process, unquestionably goes on, although naturally at a slower rate than at a higher temperature. In other words, the rate at which curdling is produced is not necessarily to be taken as a measure of the probable rate of proteolytic action on the proteids of the milk. The two processes are, without doubt, wholly independent, and in the case of predigesting milk, where naturally a smaller proportion of papoid is used than in the above experiments, the necessary digestion is accomplished without any accompanying separation of the casein; whether it be carried on at a low temperature, or at a temperature of 45° C.; and in the presence of water alone, or in the presence of sodium bicarbonate, or lime water.

Hence, in order to curdle milk, fresh or boiled, a fairly large proportion of papoid must be employed, while for the partial digestion of milk a far smaller proportion will accomplish the desired result, and that without necessarily causing any preliminary separation of the casein. From this we may infer either that the proteolytic ferment is present in larger quantity in papoid than the rennet-like ferment, or else that it is far more active than its neighbor, the milk-curdling ferment.

In predigesting milk with papoid, or any other ferment, the main object sought is the partial digestion of the casein; this being, as is well-known, the most important proteid of milk, and at the same time the one most liable to cause trouble in the feeding of infants, and others, with weak digestion. In order to test the digestive action of papoid on this proteid, the casein was separated from fresh milk by precipitation with dilute acid, partially purified by re-solution in alkaline water, and reprecipitation with acid. It was then washed with water, and though still containing some adherent

fat was reasonably pure. A series of digestions with papoid was then made in the manner already described; i. e. each digestive mixture contained 0·5 gram papoid, 8 grams of the moist casein, and 25 c. c. of water with the necessary amounts of sodium bicarbonate, etc., to give the indicated percentages.

The mixtures were warmed at 40–45° C. for 6 hours, and when ready for filtration each was made as near neutral as possible, in order to precipitate any dissolved casein not converted into products soluble in water.

The 8 grams of moist casein contained 2·1377 grams of dry proteid (dried at 110° C.). Following are the results obtained:

Reaction.		Weight of undigested residue.	Casein digested.*
Neutral		1·5128 grams	29·2 per cent.
1·0 per cent.	Bicarb. soda	1·2205 "	42·9 "
2·0 "	"	1·2463 "	41·7 "
4·0 "	"	1·3615 "	36·3 "
25·0 "	Lime water	1·2575 "	41·1 "
0·1 "	Hydrochloric acid	1·6774 "	21·0 "

It is thus evident that papoid is able to digest precipitated casein under all the above conditions, but that, as with other proteids, digestion proceeds to the best advantage in the presence of 1–2 per cent. sodium bicarbonate. It is also to be noted that lime water constitutes a particularly good medium for the digestive action of the ferment on casein.

The digestive action of papoid on milk-casein was next tested in a somewhat different manner, milk itself being used instead of the precipitated casein. In this series of experiments, each digestive mixture had a total volume of 100 c. c., composed of 25 c. c. of fresh milk† and 75 c. c. of water,‡ the latter containing 0·5 gram of papoid, and in some cases the requisite amount of sodium bicarbonate to give the indicated percentages. The solutions were warmed at 45° C. for 6½ hours, when the undissolved casein was filtered off, washed, dried and weighed. In all of these mixtures, the papoid

* The presence of adherent fat in the casein without doubt introduced some slight errors in the above results, as it was noticed when the mixtures were filtered, that some butyric acid had been developed, thus changing, for example, the reaction of the neutral solution to a distinctly acid one; but it is safe to assume that the above percentages at least approximately represent the rate of proteolytic action, under the given conditions.

† In one mixture boiled milk was used, as noted.

‡ In one mixture water and lime water, as indicated.

produced a separation of the casein inside of an hour, and in much the same order of time as shown in a previous experiment; the neutral solution of boiled milk curdling within 5 minutes, while the mixture containing the smallest percentage of sodium bicarbonate curdled last, viz: in 55 minutes. In each case, the initial separation of the casein appeared quite complete, although of course some little allowance must be made for possible error in this direction. The digestive action of papoid was, however, quite apparent to the eye, the precipitated casein visibly diminishing in amount as the digestion proceeded.

The 25 c. c. of milk yielded by precipitation with dilute acid (0·2 per cent. hydrochloric) 1·6143 grams of casein dried at 110° C. Following are the results obtained:

Reaction.	Weight of undigested casein.	Casein digested.
Neutral	0·9328 gram	42·2 per cent.
" (Boiled milk)	1·0311 "	36·1 "
0·5 per cent. Bicarb. soda	0·9868 "	38·8 "
1·0 " "	0·7473 "	53·7 "
2·0 " "	0·6979 "	56·7 "
10·0 " Lime water	1·2799 "	20·7 "

From these results, it is to be noted that while boiled milk in a neutral solution is more quickly curdled by papoid than fresh milk, digestion of the precipitated casein is somewhat less rapid. Further, in close agreement with the results found for precipitated casein, it is seen that digestion is most vigorous in the presence of 2·0 per cent. sodium bicarbonate. Somewhat peculiar, however, is the result obtained in the presence of lime water. Digestion in this case appears to have been remarkably slow; certainly not at all in conformity with the previous results with precipitated casein, and other proteids.

It is evident, however, from all of the previous results, that papoid, especially in the presence of sodium bicarbonate, is particularly well adapted for predigesting milk, the casein being converted by it, as by alkaline trypsin solutions, into soluble and more or less diffusible products.

6.—*Action of Papoid on Starch.*

In addition to the two ferments already described, viz: the proteolytic and rennet-like ferments, there is apparently present in papoid a third ferment; an amylolytic one, capable of exerting some action upon boiled starch. At all events, papoid added to

starch paste, preferably in the presence of sodium bicarbonate at 40° C., slowly converts a portion of the starch into soluble starch, and into a more soluble dextrin. This reaction, though plainly recognizable by the iodine test, is neither rapid nor very pronounced. It is not at all comparable in intensity to the proteolytic action, but still it does exist and implies the presence of a starch-converting ferment.

The best result is obtained in the presence of 2–4 per cent. sodium bicarbonate. A neutral solution of the ferment is also active, but even 0·05 per cent. hydrochloric acid will produce marked inhibition. In the latter case, however, the ferment is not destroyed, but simply checked in its action, since neutralization of the acid fluid with sodium bicarbonate (or better, making it alkaline) is followed by a renewal of the amylolytic action. Experimentally, the most satisfactory method of demonstrating the starch-converting power is to use a mixture composed of 0·5 gram papoid and 25 c. c. of a 1·0 per cent. starch paste, in which is dissolved 0·5 gram sodium bicarbonate.

So far as the writer's experience extends, the amylolytic action is limited to the conversion of starch into soluble bodies giving little or no color with iodine, ordinary soluble starch being first formed. Little or no reducing sugar appears.

7.—*Probable Action of Papoid in the Body.*

Experiments already recorded show that papoid is active in the presence of percentages of sodium carbonate far larger than normally occur in any of the secretions found in the alimentary tract. In fact, in the presence of 0·5 per cent. sodium carbonate, the reputed average strength of the pancreatic juice, the proteolytic action of papoid is slightly increased. The question naturally suggests itself, however, in this connection, whether the alkaline pancreatic juice might not digest and destroy papoid, thus checking effectually the latter's action. This important question was answered by trying several experiments, one of which may be profitably reported. An artificial pancreactic juice was prepared by warming at 40° C. 1 gram of trypsin (Fairchild's) with 100 c. c. of a 1·0 per cent. sodium bicarbonate-solution and filtering from the undissolved residue. With this solution, two digestions were made with cooked beef proteids; one with 25 c. c. of the prepared trypsin solution alone, the other with the same quantity of trypsin solution plus 0·5 gram papoid.

The two mixtures were warmed at 40° C. for 6 hours. The 10 grams of cooked beef proteids contained 3·5707 grams of dry proteid (110° C.).

Medium.	Weight of undigested residue.	Proteid digested.
Trypsin sol. alone	1·5657 grams	56·1 per cent.
" " with papoid	1·1041 "	69·0 "

From these results it is evident that the two proteolytic ferments, trypsin and papoid, can work together in the same solution, the latter ferment contributing to the digestive strength of the former. At first glance, it might seem that in combining trypsin and papoid we should have, providing there is no destruction of the latter ferment, a proteolytic action numerically equivalent to that of the two ferments, but the concentration of the solution must be taken into account, as well as the inhibitory effects of the accumulated products of digestion, both of which cannot well help acting as a check to continued ferment action. Doubtless, with a weaker trypsin solution the digestive action of papoid would be more strongly marked.

Another point to be taken into account in considering the action of papoid in the intestine, is the influence of bile. Two experiments were therefore tried; one with ox bile, the other with human bile obtained from a fistula.

The 10 grams of raw beef proteids used in the first experiment contained 2·707 grams of dry proteid (110° C.).

The mixtures were warmed at 45° C. for 7 hours.

Dry ox bile.	Weight of undigested residue.	Proteid digested.
0 (Neutral)	0·9043 gram	66·5 per cent.
4·0 per cent.	1·1198 "	58·6 "
Dry human bile.		
0 (Neutral)	1·1070 "	59·6 "
2·0 per cent.	0·9295 "	66·1 "

In the latter experiment, with human bile, the mixtures were kept at 45° C. for 8 hours, and the 10 grams of prepared raw beef contained 2·743 grams of dry proteid (110° C.).

The human bile was quite strongly alkaline, which probably accounts, in part, for the increased digestive action noticed. Both results, however, clearly show that the presence of bile offers little or no obstacle to the action of papoid in the intestinal tract.

The only remaining point to be considered is the probable fate of papoid in the stomach. In this connection, it has already been demonstrated that the ferment is only slightly inhibited in its action by the presence of 0·1 per cent. hydrochloric acid, and that even in the presence of 0·2 per cent. hydrochloric acid it exhibits a fair

degree of activity; both of which results clearly favor the action of papoid in the stomach.

Further, while the presence of 0·1 per cent. hydrochloric acid lessens somewhat the action of the ferment, the latter is not destroyed; hence, by neutralization of the acid the inhibitory effect is overcome and the ferment springs into renewed activity when brought in contact with an alkaline medium. By long-continued warming of the bare ferment with 0·2 per cent. hydrochloric acid, there is a pronounced destructive action. This destruction, however, is accomplished by the *free* acid; free, because of the lack of proteids present for it to combine with, a condition of things not so liable to occur in the case of a full stomach, which is naturally the time when a digestive ferment would be administered.

The above statements are illustrated by the following facts: Two portions of papoid, 0·5 gram each, were warmed at 40° C. for 5½ hours with 25 c. c. of 0·2 per cent., and 0·1 per cent. hydrochloric acid, respectively. The two solutions were then neutralized and eventually made slightly alkaline with sodium bicarbonate. Their digestive action was then compared with that of a like amount of fresh papoid, dissolved in the same quantity of fluid and of the same reaction.

The 10 grams of cooked beef proteids contained 3·4420 grams of dry proteid (110° C.).

The mixtures were warmed at 40° C. for 6 hours.

Conditions.	Weight of undigested residue.	Proteid digested.
Fresh papoid	1·7930 grams	47·8 per cent.
Previously warmed with } 0·1 per cent. HCl	1·8425 "	46·4 "
" " 0·2 " "	2·9260 "	14·9 "

It is thus evident that, under the above conditions, 0·1 per cent. hydrochloric acid may hinder the digestive action of papoid, but will not destroy the ferment. With 0·2 per cent. hydrochloric acid, however, when the acid is free and not combined with proteid matter, there is a marked destruction of the ferment; not complete, but doubtless sufficient to interfere somewhat with its action. In the presence of an excess of proteid matter, this destructive action is not so marked.

The presence of pepsin does not appear, materially, to modify the action of the dilute hydrochloric acid on papoid. I do not think that gastric juice of a given acidity has any more deleterious effect on the ferment, than acid of the same strength alone. In any event,

papoid will certainly exhibit marked proteolytic action in the presence of 0·1 per cent. hydrochloric acid and pepsin, although the conditions may not be favorable for the best action of pepsin.

This is illustrated by the following experiments: an artificial gastric juice was prepared by dissolving some commercial pepsin (Fairchild's) in 0·1 per cent. hydrochloric acid, in the proportion of 0·1 gram pepsin to 25 c. c. of acid. Digestions were then made, with and without papoid, of raw and cooked beef proteids, with the following results:

The 15 grams of raw beef used contained 4·0494 grams of dry proteid, while the 10 grams of cooked beef contained 3·9608 grams of dry proteid (110° C.).

The digestions were kept at 40° C. for 6 hours.

a. With raw beef proteids.

Character of the fluid.	Weight of undigested residue.	Proteid digested.
25 c. c. pepsin-HCl alone	3·3626 grams	16·9 per cent.
" " " + 0·5 gram papoid	1·4822 "	63·3 "

b. With cooked beef proteids.

Character of the fluid.	Weight of undigested residue.	Proteid digested.
25 c. c. pepsin-HCl alone	3·8689 grams	2·3 per cent.
" " " + 0·5 gram papoid	3·2124 "	44·1 "

The action of papoid is prominent here simply because the conditions are favorable for its action, while they are not well adapted to the action of pepsin. The two points to be emphasized are, however, first, that the presence of pepsin does not interfere with the action of papoid in an acid medium, where the other conditions are favorable to the latter ferment; and secondly, that any direct comparison of the digestive action of the two ferments cannot well be made, since they act under such totally different conditions as regards dilution, reaction, etc. Further, in studying the action of acids on any ferment we have to consider not only the influence of a given percentage of acid, but must also take into account the absolute amount of acid, both in proportion to a given quantity of ferment and the proteid matter to be digested. It is hardly necessary to detail the several experiments that have led to this conclusion; one alone will suffice. The following mixtures were warmed at 40° C. for 6 hours, with 10 grams of cooked beef proteids, with results as indicated:

Digestive mixture.					Proteid digested.
100 c. c. 0·1 per cent. HCl,	0·1 gram pepsin,	0	papoid		21·4 per cent.
" "	" "	0·5 gram "			21·3 "
50 c. c. 0·1 per cent. HCl,	0·1 gram pepsin,	0	papoid		8·2 per cent.
" "	" "	0·5 gram "			14·8 "
25 c. c. 0·1 per cent. HCl.	0·1 gram pepsin,	0	papoid		6·8 per cent.
" "	" "	0·5 gram "			34·7 "

It is thus seen that 0·5 gram of papoid, acting upon 10 grams of cooked beef proteids, in the presence of pepsin and 0·1 per cent. hydrochloric acid, will digest a reasonable amount of the proteid when the volume of the acid fluid is only 25 c. c., the pepsin action being very slight. As the volume of acid fluid is increased to 50 c. c., then the papoid action diminishes almost 50 per cent., while the pepsin action shows a slight increase. Increasing the volume of acid fluid still further, to 100 c. c., the pepsin action becomes paramount. Hence, it is evident that the rate of action of papoid in the stomach hinges mainly upon the presence or absence of an excess of *free* acid. With only combined hydrochloric acid present, and an excess of proteid matter and salts, a condition of things generally prevalent especially in the early stages of digestion, papoid cannot well help exerting its peculiar proteolytic power. And in this connection, it is to be remembered that papoid acts to the very best advantage in a concentrated fluid, in the presence of an excess of proteid matter.

From the foregoing experiments, the following conclusions may be drawn :

1. That papoid is a true, soluble, digestive ferment or mixture of ferments, of vegetable origin.

2. That it has marked proteolytic action in acid, alkaline, and neutral solutions and in the presence of many chemicals, antiseptics, and therapeutic agents.

3. That it has a peculiar softening and disintegrating action on proteids, and that its general proteolytic action is that of a genuine digestive ferment, similar to the ferments of animal origin.

4. That it has a certain amount of amylolytic, or starch-dissolving power.

5. That it has a marked rennet-like action upon milk, and a pronounced digestive action upon milk-casein.

6. That it exerts its peculiar digestive power at a wide range of temperatures.

7. That the ordinary conditions of health and disease in the stomach and intestine are not liable to check its action, while certain possible conditions may accelerate it.